Paper # N-089

CHARACTERIZATION OF *DESULFOVIBRIO VULGARIS* GROWTH IN EXTREMOPHILE TURBIDOSTAT REACTORS

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Abstract

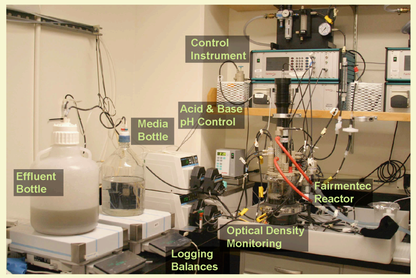
We are studying the response of sufate reducing bacteria Desulfovibrio vulgaris, an obligate anaerobe, to a range of environmental stressors (such as a salt, pH change or oxygen). One method of performing these tests is to grow the bacteria in turbidostats to a steady-state concentration, as controlled by the dilution rate, or flow rate through the reactor before applying the stress. Desulfovibrio vulgaris is grown in a defined medium for sulfate reducers in FairMenTec reactors (Bioengineering, Switzerland). The reactors are designed for growth of extremophiles, and are constructed of all non-metallic materials. The reactors are equipped with a temperature controlled water jacket and a pH control system, and a controllable agitator. The reactor is inoculated with 10% culture, and grown at 30°C until cell density has increased to the desired level. pH is maintained at 7.2. Sterile media is then pumped at a constant rate to the reactor, and effluent is continuously withdrawn from an overflow tube to maintain a constant reactor volume. The reactor is purged with nitrogen gas to maintain anaerobic conditions. The reactor is periodically sampled for cell density (by optical density at 600 nm, and AODC) and protein concentrations. A dilution rate of 0.25 1/h produces cells in log phase (low 1x108 cells/ml), that are macroscopically well-dispersed. At lower dilution rates, when cell concentrations are near 1x109 cells/ml and are in stationary phase, the biomass clumps into large flocs. At low dilution rates, and a pH below 7, the biomass flocs were less dense, and the reactor broth light-brown in color. At this pH, the reduced sulfate is mostly H2S, which is stripped from the reactor by the nitrogen purge gas. At a higher pH (7.2), the broth color darkened, and the biomass flocs were denser and more dendritic - like threads of FeS with adhered biomass. Under log phase conditions, where the biomass was macroscopically well-dispersed, we observed microscopic clusters of cells. The effect of the clusters could be seen in the shift in the correlation between optical density (absorbance at 600 nm) and cell density when the reactor stir-rate was increased from 125 RPM to 150 RPM. Biomass flocculation, and the characteristics of the floc, are dependent upon biomass age and environmental factors. This system can be used for large scale biomass production of steady-state obligate anaerobe and extremophile bacteria.

Introduction

This work is part of a multi-institutional investigation of the molecular response of microorganisms to environmental stressors, in order to understand their potential for heavy metal and radionuclide remediation in groundwater aquifers. In this poster, we describe our study of turbidostat reactors to produce biomass for a suite of molecular analyses, using sufate reducing bacteria *Desulfovibrio vulgaris,* an obligate anaerobe. Our objective is to determine the operating parameters that will produce the maximum cell density in log-phase growth. Turbidostats may provide more reproducible cell populations in terms of their growth phase, as compared to our current production method in batch reactors (see Huang et al., Poster Presentation Number N-170, Session #228).

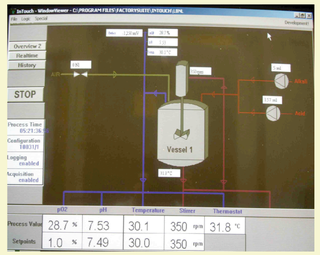
Experiments

Bioreactor System



This photograph shows a 2 L FairMenTec reactor (Bioengineering, Switzerland), the control instrumentation, sterile media feed and effluent container. Flow rates are monitored with logging balances. The reactor is periodically sampled for cell density (by optical density at 600 nm, and AODC) and other analyses (protein, PLFA, organic acids, inorganic ions.) Continuous optical density monitoring of recycle line is being tested.

Reactor Control Screen



Software provided with FairMenTec allows control of setpoints from a PC, and continuous logging of temperature, pH and stir-rate . (This screen shows a pH of 7.5, which is offset from the actual pH of 7.2. Also, the pO2 indicator is incorrect; dissolved O2 values are actually near zero.)

Turbidostats and Dilution Rates

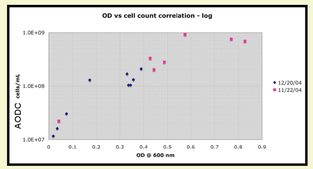
In a turbidostat, cell density is controlled by the residence time of the cells in the reactor. Nutrients are supplied in excess, so that they do not limit growth, and the flow rate through the reactor washes out the culture at the rate at which it accumulates. The residence time of the cells in the well-mixed reactor is equal to the reactor volume, V, divided by the flow rate F. The dilution rate, D, is equal to the inverse of the residence time, or, D = F / V; in a fixed-volume reactor, the flow rate controls the dilution rate, and therefore cell density.

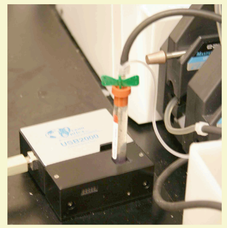
Procedures

- Inoculum is grown from -80 C freezer stock in LS4D media in anaerobic chamber
- The reactor is autoclaved with LS4D media (sulfate/lactate) and cooled on bench with nitrogen gas purge
- After cooling, vitamins and reductant (4% titanium citrate) and inoculum are injected to reactor
- Culture grows in batch mode until optical density of ca 0.4 (log-phase cells)
- Sterile media (autoclaved, cooled, amended with vitamins and reductant, with nitrogen headspace) is delivered to the reactor at a constant flow rate

Experiments (cont'd)

Optical Density Monitoring



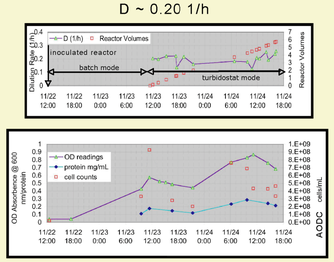


Cell density is correlated with the absorbance of 600 nm wavelength light through the sample, as shown on the left. This measurement is referred to as optical density. This is a key monitoring parameter to determine growth rates and fluctuations. We are currently testing continuous monitoring of OD, by running a recycle line from the turbidostat through a small spectrophotometer (USB 2000, Ocean Optics). The assembly is shown on the right. Ultimately, the reactor flow rate could be controlled by the change in cell density.

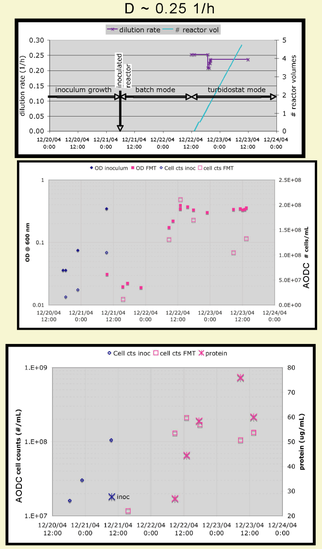
Results

Determination of dilution rate: At steady-state, the growth rate equals the dilution rate. In batch reactors, we have measured a five hour doubling time, which is equivalent to a growth rate of 0.14 1/h. To maintain cells in log phase, the dilution rate should therefore be >= to 0.14 1/h. We have measured a range of growth rates in turbidostats from 0.12 to 0.21 1/h, as a function of various operating parameters (e.g., stir-rate, temperature, pH, gas purging). The following results show the relationship between cell density and dilution rate in two test-runs.

D ~ 0.20 1/h



D ~ 0.25 1/h



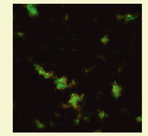
All indicators of biomass concentrations (AODC cell cts, ODs and protein concentrations) were well correlated. Small changes in flow rate have a large effect on biomass concentrations. It is generally assumed to take several reactor volumes to reach steady-state. although we intend to verify that.

The lower dilution rate (on the left) produces cell counts that were twice as high, and protein concentrations that were 2 to 3 times greater compared to the higher dilution rate (shown on the right). The next tests will be run for a dilution rate of 0.15 1/h.

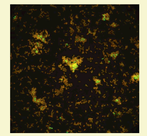
Results (cont'd)

Microscopic Observations

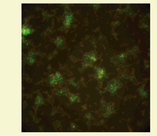
Live/Dead Stain - cell shock and recovery from injection to reactor



inoculum just before injection into reactor: high porporation of live cells

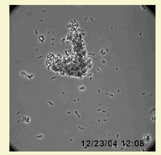


after injecting inoculum into reactor: higher proportion of dead cells clumped around live cells; we observed more frothing in reactor which may indicate stress response.

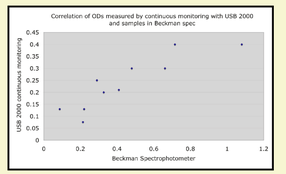


after one reactor volume of flow - great increase in fraction of live cells, no frothing observed during this time.

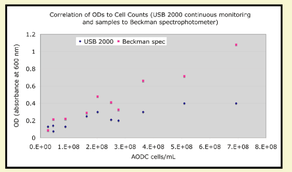
Wet Mounts - cell morphology and iron sulfide particles - cell aggregates occur around iron sulfide particles (bright spots)



Optical Density Monitoring



Absorbance measured by continuous monitoring of the reactor recycle line through the USB 2000 correlate with 1 mL samples taken from the reactor and measured in a Beckman spectrophotometer.



Both absorbance measurements correlate with AODC, however the correlation falls off in the USB 2000 at cell counts greater than 4E8 #/mL. This may be due to inadequate mixing in the recycle line; the higher cell concentrations may indicate approach to stationary phase and increased aggregation of cells.

Conclusions

- Turbidostat reactors can be used to produce high biomass density extremophile cultures
- Need to understand culture behavior during approach to steady state (first few reactor volumes of flow)
- Cell aging (approach to stationary phase growth) affects OD-cell density correlations because of increased flocculation
- Biomass density very sensitive to dilution rate in reactor; optimization and control of dilution rate are essential
- Optical density monitoring shows promise for control of turibidostat reactors

Acknowledgement

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